Docket No.: 0230-0160P
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Kenji ASANO et al.

Application No.: 09/856,716 Confirmation No.: 3342

Filed: January 28, 2002 Art Unit: 1642

For: LAK ACTIVITY-SCREENING MATERIALS CONTAINING LENTINUS EXTRACT OF EDODES MYCELIUM AND LAK ACTIVITY-SCREENING METHODS USING THE EXTRACT

Examiner: L. Yao

APPEAL BRIEF

MS Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

As required under § 41.37(a), this brief is filed two months after the Notice of Appeal filed in this case on November 5, 2007, and is in furtherance of said Notice of Appeal.

The fees required under § 41.20(b)(2) are dealt with in the accompanying TRANSMITTAL OF APPEAL BRIEF.

This brief contains items under the following headings as required by 37 C.F.R. § 41.37 and M.P.E.P. § 1205.2:

I.	Real Party In Interest
II	Related Appeals and Interferences
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(I) *REAL PARTY IN INTEREST*

The Real Parties in Interest of the present application are:

1) KOBAYASHI PHARMACEUTICAL CO., LTD, Osaka, Japan; and

2) HITOSHI NAGAOKA, Chiba, Japan

(II) *RELATED APPEALS AND INTERFERENCES*

There are no related appeals or interferences.

(III) *STATUS OF CLAIMS*

Claims 1 and 3-4 are pending in the application. Claim 2 has been cancelled. Claims 3-4 are withdrawn. Claim 1 is rejected and is appealed herein.

(IV) *STATUS OF AMENDMENTS*

No amendments to the claims were made subsequent to final rejection. The response filed on October 29, 2007, containing only Remarks, has been entered for consideration. See the Advisory Action of November 19, 2007, which indicates entry of the response of October 29, 2007.

(V) *SUMMARY OF CLAIMED SUBJECT MATTER*

The instant invention of claim 1 is directed to an *in vitro* method of determining whether a particular extract of *Lentinus edodes* mycelium is active at enhancing the activity of lymphokine activated killer (LAK) cells of a patient. (See page 6, lines 16-21.)

The method of the invention requires the following steps:

a) isolating peripheral blood from a subject;

b) preparing a LAK-induced sample by treating a lymphocyte fraction obtained from the blood with an extract of *Lentinus edodes* mycelium and preparing a control sample of the lymphocyte fraction, which has not been treated with the extract; and

c) measuring and comparing the LAK activity of the lymphocyte fraction treated with the extract and that of the control sample. (See page 7, lines 1-15 of the specification.)

The claimed method further requires that the extract from the *Lentinus edodes* mycelium be prepared in a specific way, which results in a specific extract. The extract used in the invention is required to be prepared by first crushing and delignifying a solid medium, which is based on bagasse and defatted rice bran and which contains the *Lentinus edodes mycelium,* in the presence of water and one or more additive enzymes selected from cellulose, protease, and glucosidase, so as to prepare a suspension. Then the temperature of the suspension is raised to a sufficient temperature to inactivate the enzymes. (See page 9, lines 11-26 of the specification.)

(VI) *GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL*

The sole issue on appeal is whether the subject matter of claim 1 is obvious under 35 U.S.C.§103 over the disclosure Yamamoto et al. (Biosci. Biotechnol. Biochem. **61**:1909-1912 (1999)) (hereinafter referred to as "Yamamoto et al.") combined with the disclosure of Mizoguchi et al. (Gastroenterol. Jpn. **22**:459-464 (1987)) (hereinafter referred to as "Mizoguchi et al.").

(VII) *ARGUMENT*

1) The instant invention

As discussed above, the present invention, as recited in claim 1, is directed to an *in vitro* method of determining whether a particular extract of *Lentinus edodes* mycelium is active at enhancing the activity of lymphokine activated killer (LAK) cells of a particular patient/subject

The method of the invention requires the following steps:

a) isolating peripheral blood from a subject;

b) preparing a LAK-induced sample by treating a lymphocyte fraction obtained from the blood with an extract of *Lentinus edodes* mycelium and preparing a control sample of the lymphocyte fraction, which has not been treated with the extract; and

c) measuring and comparing the LAK activity of the lymphocyte fraction treated with the extract and that of the control sample. (See page 7, lines 1-15 of the specification.)

However, the claimed method further requires that the extract from the *Lentinus edodes* mycelium be prepared in a specific way, which results in a specific extract. The extract used in the invention is required to be prepared by first crushing and delignifying a solid medium, which is based on bagasse and defatted rice bran and which contains the *Lentinus edodes mycelium,* in the presence of water and one or more additive enzymes selected from cellulose, protease, and glucosidase, so as to prepare a suspension. Then the temperature of the suspension is raised to a sufficient temperature to inactivate the enzymes.

It is well-known in the fields of immunology and oncology that many tumor cells express tumor specific antigens. One therapy for treating various tumors involves the specific immuno-targeting of the tumor cells through the tumor antigens. It has been further shown that incubating peripheral lymphocytes with interleukin 2 (IL-2) induces the production of a set of killer cells that can target and kill a wide range of cancer cells, including cancer cells that are resistant to killing by natural killer cells (NK cells). These IL-2 induced killer cells are called "lymphokine activated killer" cells or LAK cells.

One immunotherapeutic approach with LAK cells is an "adoptive immunotherapy" wherein the peripheral lymphocytes of a patient are treated with IL-2 *in vitro* to induce LAK cells and then the LAK cells that show anti-tumor activity *in vitro* are reinfused back into the patient. However, this adoptive immunotherapy has very differential efficacy from patient to patient, with reasonably good success in some patients to virtually no effect at all in other patients. This form of adoptive immunotherapy with LAK cells also has several drawbacks, particularly in view of the widely varying efficacy between patients. The therapy is extremely stressful on the physical state of the patient first because of the stress involved with isolating a sufficient number of leukocytes from the patient and secondly because of the side effects associated with the presence of the IL-2. Adoptive immunotherapy with LAK cells and IL-2 causes particularly harsh side effects, including general prostration, chills, fever, hypoalbuminemia, anemia, eosinophilia etc. This adoptive immunotherapy is also extremely expensive due to the high costs associated with isolating and culturing the lymphocytes.

Given the draw backs associated with LAK adoptive immunotherapy it would be ideal if a doctor could determine the efficacy of the treatment with a particular patient before putting the patient through the physical stress and incurring the costs.

Lentinus edodes is a common edible mushroom (Shitake mushroom), which has also been recently studied for its pharmacological properties. For example, *Lentinus edodes* was shown in the 1980's to have some antitumor activity.

The present inventors have found that *Lentinus edodes* possesses a LAK enhancing activity that is independent of IL-2 exposure. Previously, it was shown that treating isolated lymphocytes with *Lentinus edodes* and then reinfusing the activated LAK cells into a patient enhances the LAK activity. This approach has the advantage over the traditional IL-2 induced LAK immunotherapy of avoiding the side effects associated with the IL-2. However, this approach has some similar draw backs to those associated with IL-2 activation of LAK cells, specifically, the draw backs of the stress on the patient in isolating the lymphocytes and the costs for the isolation and culturing of the lymphocytes. Thus, as with IL-2 activated LAK cell adoptive immunotherapy, it would be ideal if the efficacy of the immunotherapy could be determined prior to subjecting the patient to the full lymphocyte isolation procedures and incurring the costs.

The present inventors have found that it is possible to screen an extract of *Lentinus edodes* mycelium *in vitro*, for LAK-enhancing activity. The inventors have further found that if an extract is prepared in accordance with the recited method, a product results whose efficacy *in vitro* correlates to the activity *in vivo*. Thus, the inventors have developed an *in vitro* method for screening a specifically prepared extract of *Lentinus edodes* mycelium to determine whether that extract will be efficacious for a particular patient in LAK activity enhancement. The method of the invention requires that the extract used in the screening method be prepared in a specific manner. Specifically, as recited in claim 1, the extract is prepared by first crushing and delignifying a solid medium, which is based on bagasse and defatted rice bran and which contains the *Lentinus edodes mycelium,* in the presence of water and one or more additive enzymes selected from cellulose, protease, and glucosidase, so as to prepare a suspension. Then the temperature of the suspension is raised to a sufficient temperature to inactivate the enzymes.

The inventors have found that an extract prepared using mycelium raised in the recited manner and further digested with the recited enzyme(s) results in a preparation, whose *in vitro* LAK enhancing activity correlates with its *in vitro* LAK enhancing activity. Thus, the extract can be successfully used in the recited screening method.

2) The prior art teachings

The outstanding rejection is well summarized in the Office Action issued on May 4, 2007. The Examiner relies on the combined teachings of Yamamoto et al. and Mizoguchi et al. Briefly, the Examiner asserts that Yamamoto et al. teach a method of preparing NK lymphocytes, and measuring the LAK activity of the NK cells treated with a fraction from *Lentinus edodes* mycelium. The Examiner further asserts that Yamamoto et al. differs from the instant invention only in the recited method of how the extract from the *Lentinus edodes* mycelium is produced.

The secondary reference of Mizoguchi et al. is asserted to make up for this deficiency of Yamamoto et al. by teaching the claimed feature of preparing the extract by crushing an delignifying the mycelium, which are in a solid medium of bagasse and defatted rice bran, and digesting the mycelium with mycelia enzymes.

Thus, Yamamoto et al. is asserted to teach steps a) through c) of claim 1, while Mizoguchi et al. is asserted to teach the feature of the extraction process of claim 1. Appellants respectfully disagree with the Examiner's interpretation of the disclosures of both Yamamoto et al. and Mizoguchi et al.

a) Yamamoto et al. -

Yamamoto et al. considered the *in vitro* immunopotentiating effects on certain cell types and antiviral activity of a water-soluble lignin rich fraction of *Lentinus edodes* mycelium called "JLS-18". See page 1909, left column of Yamamoto et al. In Yamamoto et al., the *Lentinus edodes* mycelium extract is prepared by culturing the mycelia on a solid medium of mainly bagasse and then extracting with hot water followed by filtration and separating the *Lentinus edodes* mycelium into three fractions using ultrafiltration. The fraction from the ultrafiltration

having a molecular weight between 3 x 10^4 to 1 x 10^6 daltons was further purified using hydrophobic column chromatography. The fraction obtained from the column was the JLS-18 fraction. See page 1909, *"Preparation of JLS-18"*. Yamamoto et al. then looked at the effects of the JLS-18 on NK cells, macrophages, a mixed lymphocyte assay and IL-6 production. (See page 1910, left column.) The data presented in Yamamoto et al. looked at the activity of *Lentinus edodes* mycelium and the JLS-18 fraction.

The Examiner is incorrect in asserting that Yamamoto et al. teaches the steps of the instant invention of claim 1 with the exception of the process by which the extract is made. Contrary to the assertion of the Examiner, Yamamoto et al. does not disclose the enhancement of LAK activity by an extract of *Lentinus edodes* mycelium.

Yamamoto et al. only discloses that the extract of the reference has an effect on activating NK cells, T cells and macrophages, and Yamamoto et al. does not contain any description as to whether the extract enhances LAK activity.

On page 4 of the Office Action of May 4, 2007, the Examiner asserts that NK cells that are isolated from a patient will have a base-line cytotoxicity against the target cells. The Examiner further asserts that activating NK cytotoxicity and enhancing LAK activity in the context of the invention are the same. However, the Examiner's position, in this regard, is technically incorrect.

As is defined in the present specification, "LAK activity" means the anti-tumor cytotoxic activity of cytotoxic T-lymphocytes, which attack tumors unrecognizable by lymphocytes having NK activity, but which have little influence on autologous normal cells (see page 7, line 28 to page 8, line 2 of the specification). The definition in the present specification demonstrates that "LAK activity" was considered in the art as being a different bioactivity from "NK cytotoxicity" at the filing date of the present application.

Further, the present specification defines that "LAK activity-enhancing" refers to the effect of enhancing this LAK activity, that is, inducing the production of LAK cells from lymphocytes or further enhancing the antitumor activity of existing LAK cells. On the other hand, "activating NK cytotoxicity" as mentioned by the Examiner is to increase cytotoxicity of NK cells against tumors which are recognizable and attacked by NK cells.

Thus, since it is generally considered by those skilled in the art that the LAK cells are a different population having different bioactivity from NK cells and that the target of "LAK activity" is completely different from that of "NK cytotoxicity", the Examiner's assertion is not correct and Yamamoto et al. fails to disclose any enhancement of LAK activity.

b) Mizoguchi et al. -

Mizoguchi et al. discloses the *in vitro* effects of a preparation from *Lentinus edodes* mycelium on preventing immunological (i.e. antibody-dependent cell-mediated and macrophage-mediated) liver cell damage. Appellants note for the record, that while the Examiner cites to page "628" of Mizoguchi et al. in the Office Action of May 4, 2007 for the alleged relevant teaching, Appellants believe that the intended section of Mizoguchi et al. appears at page 460, right column, Item 2, which describes the preparation of the *Lentinus edodes* mycelium extract. In this section Mizoguchi et al. state that:

a) the mycelium were raised in solid medium of bagasse and defatted rice bran;

b) the medium was disrupted prior to formation of fruiting bodies;

c) "The disrupted material was incubated in water at 40-50°C for 60 hours to promote autolysis of the mycelia and partial digestion of the culture medium **with mycelia enzymes. The digest was then extracted with water at 60°C.**" (emphasis added)

3) Unobviousness of the invention over the combined prior art teachings

In determining obviousness, the scope and content of the prior art should be determined, along with differences between the prior art and claims at issue and the level of ordinary skill in art. *Graham v. John Deere Co.* 383 148 USPQ 459 (US 1965). While there is no rigid test for determining obviousness, "Rejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *KSR International Co. v. Teleflex, Inc.* 82 USPQ2d 1385 (US 2007).

As discussed above, the instant invention is directed to an *in vitro* method of screening for whether or not an extract of *Lentinus edodes* mycelium will have an *in vivo* efficacy for

enhancing LAK activity for a particular patient. The instant invention requires that the extract of *Lentinus edodes* mycelium used in the *in vitro* screening method be prepared in a particular manner. Specifically, that the extract is prepared by first crushing and delignifying a solid medium, which is based on bagasse and defatted rice bran and which contains the *Lentinus edodes mycelium,* in the presence of water and one or more additive enzymes selected from cellulose, protease, and glucosidase, so as to prepare a suspension. Then the temperature of the suspension is raised to a sufficient temperature to inactivate the enzymes.

As a first point, the Examiner is incorrect in asserting that Yamamoto et al. teaches the steps of the instant invention of claim 1 with the exception of the process by which the extract is made. Claim 1 recites as step c) "measuring and comparing the LAK activity of the LAK-induced sample and the control sample **to determine *in vitro* whether the extract of *Lentinus edodes* mycelium has a LAK activity-enhancing effect suitable for the subject**" (emphasis added). Thus, step c) is indicative of the feature of the invention that the invention determines *in vitro* whether or not an extract of *Lentinus edodes* mycelium will be efficacious *in vivo* for a particular subject.

In the Office Action of May 4, 2007, the Examiner states that the feature that the instant invention takes place *in vitro* is not given patentable weight because "it does not recite an active step". The Examiner is incorrect in this position because step (c) of claim 1 clearly recites as an active step, "measuring and comparing" to a control sample to determine whether the extract has suitable a LAK-activity effect suitable for the subject. Appellants respectively note that the recitation of the active form of the verbs, i.e. "measuring" and "comparing" are indicative of an active step.

As a second point, and as discussed above, Yamamoto et al. does not disclose an enhancement of LAK activity. Rather, Yamamoto et al. only discloses that the extract of the reference has an effect on activating NK cells, T cells and macrophages, and does not contain any description as to whether the extract enhances LAK activity. As discussed above in detail, the Examiner's assertion that activating NK cytotoxicity and enhancing LAK activity in the context of the invention are the same is incorrect. As is defined in the present specification, "LAK activity" means the anti-tumor cytotoxic activity of cytotoxic T-lymphocytes, which attack

tumors unrecognizable by lymphocytes having NK activity, but which have little influence on autologous normal cells (see page 7, line 28 to page 8, line 2 of the specification). The definition in the present specification demonstrates that "LAK activity" was considered in the art as being a different bioactivity from "NK cytotoxicity" at the filing date of the present application.

Further, the present specification defines that "LAK activity-enhancing" refers to the effect of enhancing this LAK activity, that is, inducing the production of LAK cells from lymphocytes or further enhancing the antitumor activity of existing LAK cells. On the other hand, "activating NK cytotoxicity", as mentioned by the Examiner, is to increase cytotoxicity of NK cells against tumors, which are recognizable and attacked by NK cells. Thus, it is generally considered by those skilled in the art that the LAK cells are a different population having different bioactivity from NK cells and that the target of "LAK activity" is completely different from that of "NK cytotoxicity".

The present application clearly discloses for the first time that the extract of the invention can enhance LAK activity both *in vivo* and *in vitro,* and that the *in vitro* function of the extract correlates with that *in vivo*. Such differences in effects between the present invention and that of Yamamoto et al. are due to the differences between the components of the extract of the present invention and that of Yamamoto et al. as discussed below.

A third important difference in the teachings of Yamamoto et al. and the invention is the composition used in the method. If an extract is prepared as recited in claim, the resulting extract will have particular components. As shown in Example 1 of the specification, the resulting extract will be have a sugar composition of approximately, 40% glucose, 15% xylose, 8% arabinose, 8% mannose, 5% galactose, 12% glucosamine and 11% glucuronic acid.

Yamamoto et al. describes that the composition of sugars of the extract of the reference "was 70 % of xylose, 20 % of arabinose, and 8 % of glucose (data not shown)" (See page 1910, right column, second paragraph).

Comparing the sugar composition of the extract of the present invention and that of Yamamoto et al., there are at least the following differences in a sugar composition between the extract of the present invention and that of Yamamoto et al. as disclosed in the instant specification and the disclosure of Yamamoto et al.

	Xylose	Arabinose	Glucose
the present invention	~15 %	~8 %	~40 %
Yamamoto et al.	70%	20%	8%

As is clear from the comparison above, the sugar composition of the extract of the present invention is completely different from that of Yamamoto et al.

In addition, as described in Example 1 (see pages 13-14 of the present specification) the extract of the present invention contains approximately 25% (w/w) carbohydrates, 20 % (w/w) proteins and 3 % (w/w) polyphenols and "further contains 8 % crude fat, 22 % crude ash and about 20 % soluble nitrogen-free materials other than carbohydrates". On the other hand, the preparations in Yamamoto et al. contain the following components (See Table II on page 1910, left column of Yamamoto et al.):

Table II. Chemical composition of *Lentinus edodes* mycelium ("LEM") and JLS-18 of Yamamoto et al.

%	Lignin	Sugar	Protein	Ash	Other
LEM	27.4	33.9	10.4	13.5	14.8
JLS-18	76.4	21.3	2.3	N.D.	-

The differences between the components of the extract of the present invention and the components of the preparations of Yamamoto et al. are summarized in the following table.

%	Lignin	Sugar	Protein	Ash	Other
LEM of Yamamoto et al.	27.4	33.9	10.4	13.5	14.8
JLS-18	76.4	21.3	2.3	N.D.	-
the present invention	-	~25	~20	22	~31

Thus, the extract made in accordance with the process recited in claim 1, is clearly a different composition than that in Yamamoto et al., as shown with the profile of the sugars present, as well as at least the protein and ash content. Thus, by requiring that the extract be made by the recited method, it is clear that the method of the invention utilizes a different extract than that disclosed in Yamamoto et al.

The Examiner relies on Mizoguchi et al. for making up fundamental deficiency of Yamamoto et al. of the specifically recited method of making the *Lentinus edodes* mycelium extract, which results in a specific extract, as discussed above. However, Mizoguchi et al. does not teach the extraction method recited in claim 1. Thus, Mizoguchi et al. cannot combine with Yamamoto et al. to achieve the instant invention.

Appellants note for the record, that while the Examiner cites to page "628" of Mizoguchi et al. in the Office Action of May 4, 2007 for the alleged relevant teaching, Appellants believe that the intended section of Mizoguchi et al. appears at page 460, right column, Item 2.

Mizoguchi et al. states at this section that,

> The disrupted material was incubated in water at 40-50°C for 60 hours to promote autolysis of the mycelia and partial digestion of the culture medium with mycelia enzymes. The digest was then extracted with water at 60°C.

There are notable, critical differences in the teachings of the Mizoguchi et al. with regard to the recited feature in claim 1 of the process for making the extract.

a) As a first difference, the preparation of Mizoguchi et al. is digested with the mycelia enzymes, not "additive enzymes" as recited in claim 1. Nor is there any disclosure of specifically cellulase, protease or glucosidase in Mizoguchi et al., as recited in claim 1.

b) In addition, there is no disclosure in Mizoguchi et al. of heating the extract to inactivate the enzymes. Mizoguchi et al. simply teaches incubating at 40-50°C and then extracting at 60°C, a temperature at which many enzymes remain active.

The teachings of Mizoguchi et al. fail to make up for the deficiencies in Yamamoto et al., which the Examiner has herself noted, i.e. digestion with specific enzymes and heating to inactivate the enzymes. As such, the invention simply cannot be achieved by combining Mizoguchi et al. and Yamamoto et al.

As shown above with regard to the composition of the extract of Yamamoto et al. compared to the instant invention, there is a correlation between how a preparation is prepared (e.g. what enzymes are used) and the composition of the resulting product. As such, the difference between Mizoguchi et al. and the recited process of claim 1 are not insignificant and Mizoguchi et al. fails to teach the recited process of making an extract of *Lentinus edodes*

mycelium as recited in claim 1. Nor does Mizoguchi et al. compensate for the additional deficiencies of Yamamoto et al. of failing to teach the screening of LAK enhancing activity and the disclosure of Mizoguchi et al. is insufficient to render the invention obvious when combined with Yamamoto et al.

Finally, indicated above, the present invention further possesses unexpected advantages that are discussed in the specification, which make the invention unobvious over the teachings of Yamamoto et al. combined with Mizoguchi et al. The present invention is directed to a method for determining whether the extract has a LAK activity enhancing effect before administration of the extract to a patient, based on the correlation between the activity of the extract *in vitro* with that *in vivo*. Neither reference discloses or suggests this advantage; which is dependent on the recited process of making the extract of *Lentinus edodes* mycelium that is recited in claim 1. As such the invention of claim 1 is not obvious over the disclosure of Yamamoto et al. combined with Mizoguchi et al.

Following the Supreme Court decision in *KSR*, the USPTO released "Examination Guidelines for Determining Obviousness Under 35 U.S.C. 103 in View of the Supreme Court Decision in *KSR International Co.*, v. *Teleflex Inc.*" *Federal Register* Vol. 72, No. 195 57526-57533 (2007). Under the Guidelines seven "rationales" are provided for determining that an invention is obvious. Those seven rationales are:

(A) Combining prior art elements according to known methods to yield predictable results;

(B) Simple substitution of one known element for another to obtain predictable results;

(C) Use of known technique to improve similar devices (methods, or products) in the same way;

(D) Applying a known technique to a known device (method, or product) ready for improvement to yield predictable results;

(E) "Obvious to try"—choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success;

(F) Known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations would have been predictable to one of ordinary skill in the art;

(G) Some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior art reference or to combine prior art reference teachings to arrive at the claimed invention.

A review of these rationales shows that all of the rationales set forth in (A) through (E) require a reliance by the Examiner on features already known in the prior art (e.g. a known method, product, technique, solution etc.). Appellants have shown above that all of the elements of the instant invention were not known in the art at the time of the instant invention. As such, a for finding of obviousness one should look to rationales (F) and (G), which both provide for situations where elements of the invention are not necessarily explicitly known in the art. However, the Examiner has failed to meet the required showings for a finding of obviousness as provided for under rationales (F) and/or (G) of the Guidelines.

Rationale (F) states,

> F. Known Work in One Field of Endeavor May Prompt Variations of it for Use in Either the Same Field or a Different One Based on Design Incentives or Other Market Forces if The Variations Would Have Been Predictable to One of Ordinary Skill in the Art
>
> To reject a claim based on this rationale, Office personnel must resolve the *Graham* factual inquiries. Office personnel must then articulate the following:
>
> (1) a finding that the scope and content of the prior art, whether in the same field of endeavor as that of the applicant's invention or a different field of endeavor, included a similar or analogous device (method, or product);
>
> (2) a finding that there were design incentives or market forces which would have prompted adaptation of the known device (method, or product);
>
> (3) a finding that the differences between the claimed invention and the prior art were encompassed in known variations or in a principle known in the prior art;
>
> (4) a finding that one of ordinary skill in the art, in view of the identified design incentives or other market forces, could have implemented the claimed variation of the prior art, and the claimed variation would have been predictable to one of ordinary skill in the art; and
>
> (5) whatever additional findings based on the *Graham* factual inquiries may be necessary, in view of the facts of the case under consideration, to explain a conclusion of obviousness.

The Examiner has failed to articulate at least requirements (2) through (4), above. There is no articulated showing in the Office Actions that "there were design incentives or market forces which would have prompted adaptation of the known...method" (i.e. the methods of Yamamoto et al. and Mizoguchi et al.). Similarly, there is no "finding that the differences between the claimed invention and the prior art were encompassed in known variations or in a principle known in the prior art" (i.e. there is no showing that it was known in the art to use the specifically recited process of making the extract as recited in claim 1, or alternatively, that there was some known principal regarding extraction processes that would have led to the modifications necessary to achieved the recited process). Nor is there any articulated showing by the Examiner "that one of ordinary skill in the art, in view of the identified design incentives or other market forces, could have implemented the claimed variation of the prior art, and the claimed variation would have been predictable to one of ordinary skill in the art".

There is similarly no articulated support in the Office Actions for a finding of obviousness under Rationale (G), which states:

> G. Some Teaching, Suggestion, or Motivation in the Prior Art That Would Have Led One of Ordinary Skill To Modify the Prior Art Reference or To Combine Prior Art Reference Teachings To Arrive at the Claimed Invention
>
> To reject a claim based on this rationale, Office personnel must resolve the *Graham* factual inquiries. Office personnel must then articulate the following:
>
> (1) a finding that there was some teaching, suggestion, or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings;
>
> (2) a finding that there was reasonable expectation of success; and
>
> (3) whatever additional findings based on the *Graham* factual inquiries may be necessary, in view of the facts of the case under consideration, to explain a conclusion of obviousness.

The Examiner has failed to articulate a basis for at least (1) and (2), above. There is no articulated finding that there was some teaching, suggestion, or motivation, either in the Yamamoto et al. or Mizoguchi et al. or in the knowledge generally available to one of ordinary skill in the art, to modify reference teachings to achieve the invention. Nor is there any showing that there would have been a reasonable expectation of success of being able to achieve a method

wherein the *in vitro* LAK enhancing activity of an extract of *Lentinus edodes* mycelium on lymphocytes isolated from a particular subject correlates to the *in vivo* activity in the particular subject.

As such, the Examiner has not properly laid the foundation for a rejection of obviousness under 35 U.S.C.§103 and withdrawal of the rejection is respectfully requested.

4) Summary of arguments

Yamamoto et al. differs from the invention of claim 1 in the following features:

a) Yamamoto et al. fails to disclose any enhancement of LAK activity by an extract of *Lentinus edodes* mycelium. The disclosure in Yamamoto et al. pertains to an effect on NK cells, T cells and macrophages, which are different from LAK cells.

b) Yamamoto et al. further fails to disclose an *in vitro* method of determining whether an extract of *Lentinus edodes* mycelium will have LAK enhancing activity for a subject *in vivo*.

c) Yamamoto et al. fails to teach a method of using an extract prepared in accordance with the process recited in claim 1. Thus, Yamamoto et al. fails to teach the extract of *Lentinus edodes* mycelium of claim 1, which is dependent on the recited process of making it.

d) Mizoguchi et al. fails to compensate for the deficiencies of Yamamoto et al. because Mizoguchi et al. fails to teach features a)-c), above. For example, Mizoguchi et al. fails to teach an extract prepared in accordance with the recited method of claim 1.

e) Neither Yamamoto et al. nor Mizoguchi et al. teach or suggest the unexpected advantage associated with the present invention of being able to determine *in vitro* the LAK enhancing activity an extract of *Lentinus edodes* mycelium will have *in vivo* with particular subject.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact MaryAnne Armstrong, Ph.D., Reg. No. 40,069 at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.14; particularly, extension of time fees.

Dated: January 7, 2008

Respectfully submitted,

By 

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(VIII) ***CLAIMS APPENDIX***

1. A method for determining whether an extract of *Lentinus edodes* mycelium *in vitro*, has a LAK activity-enhancing effect suitable for a subject, comprising the steps of:

(a) isolating peripheral blood from the subject to prepare lymphocyte fractions,

(b) preparing a LAK-induced sample by treating the lymphocyte fractions with the extract of *Lentinus edodes* mycelium and preparing a control sample in the absence of the extract of *Lentinus edodes* mycelium, and

(c) measuring and comparing the LAK activity of the LAK- induced sample and the control sample to determine *in vitro* whether the extract of *Lentinus edodes* mycelium has a LAK activity-enhancing effect suitable for the subject,

wherein said extract of *Lentinus edodes* mycelium is prepared by a method comprising the steps of:

crushing and delignifying a solid medium containing *Lentinus edodes* mycelium in the presence of water and one or more additive enzymes selected from cellulase, protease or glucosidase, to prepare a suspension, wherein said solid medium is based on bagasse and defatted rice bran; and

raising the temperature of said suspension to sufficient temperature to inactivate the one or more enzymes.

(IX) ***EVIDENCE APPENDIX***

There is no associated evidence.

(X) *RELATED PROCEEDINGS APPENDIX*

There are no related proceedings.